

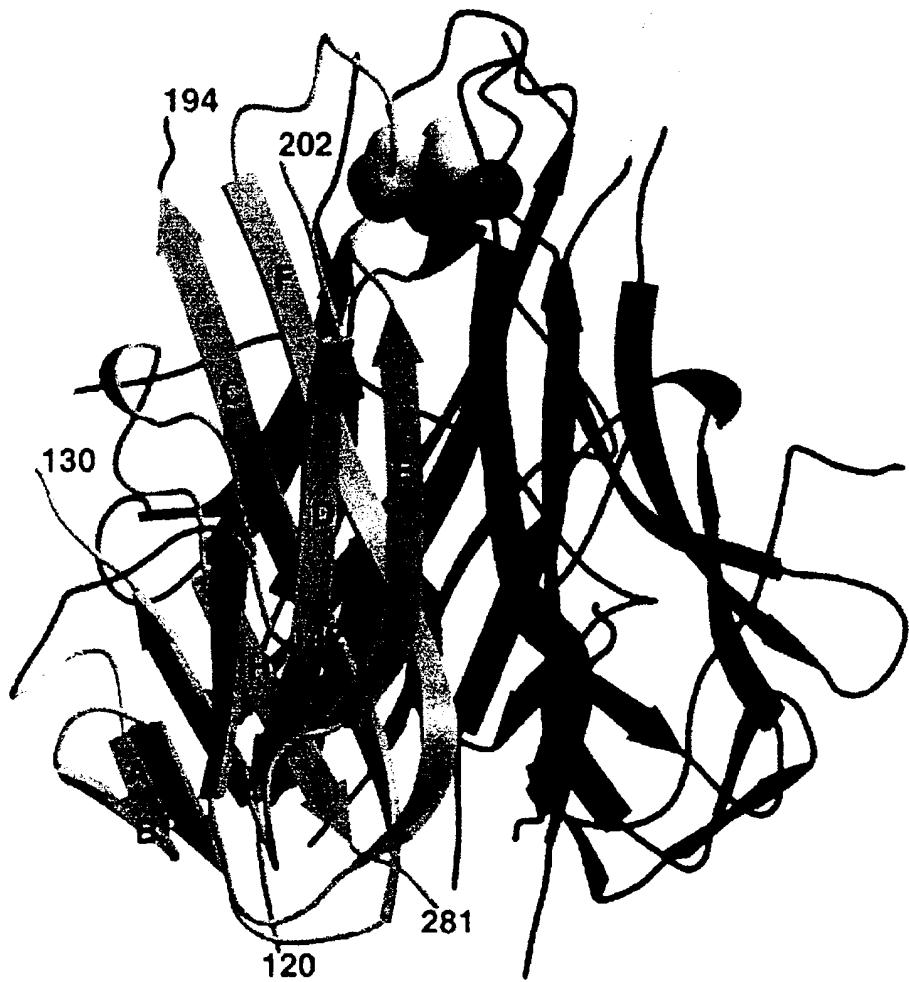
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FIG. 1

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**FIG.\_2A**

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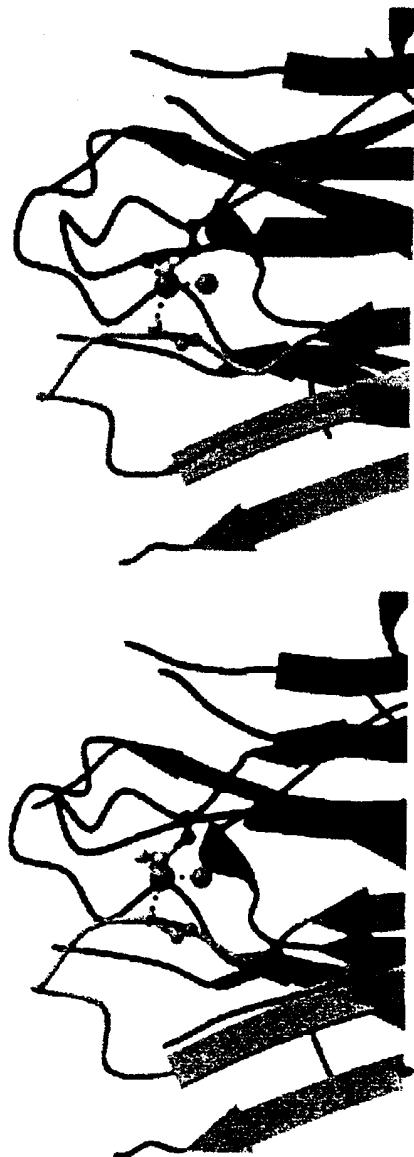


FIG.-2B



### Crystallographic Data

	<u>Apo-2L (114-281)</u>	<u>Apo-2L (91-281) D218A</u>	<u>Apo-2L (91-281) D218A</u>
Crystal			
Space Group	P6 <sub>3</sub>	R32	R32
Unit Cell (Å)	a=72.5 c=140	a=66.4 c=197.6	a=66.4 c=197.7
Resolution (Å)	3.9	1.9	1.3
Coverage (%)	94 (96)	93 (99)	100 (100)
<I/σ(I)>	5.9	10.1	12.4
# Unique (hkl)	3589	12680	41840
Redundancy	4.9	4.3	12.1
R <sub>symm</sub> (%)	15.4 (34)	6.2 (27)	6.4 (34)
# Protomers in ASU	2	1	1
Refinement			
R <sub>cryst</sub> (%)	33.8	20	
R <sub>free</sub> (%)	27.6	22	
rmsd Bonds (Å)	0.009	0.015	0.007
rmsd Angles (°)	1.79	2.0	1.41
Average B-Values	—	14	14
# Water Molecules	0	170	

$R_{\text{symm}} = \sum_{\mathbf{h}} \sum_{i} (I_{\mathbf{h}i} - \langle I_{\mathbf{h}} \rangle) / \sum_{\mathbf{h}} I$  where  $I_{\mathbf{h}}$  is the mean structure factor intensity of  $i$  observations of symmetry-related reflections with Bragg index  $\mathbf{h}$ .  $R_{\text{cryst}} = \sum_{\mathbf{h}} \sum_{i} |F_{\text{obs}} - |F_{\text{calc}}| / \sum |F_{\text{obs}}|$  where  $F_{\text{obs}}$  and  $F_{\text{calc}}$  are the observed and calculated structure factor amplitudes.  $R_{\text{free}} = \sum_{(\mathbf{h}k)} \varepsilon \tau |F_{\text{obs}}(\mathbf{h}k) - k|F_{\text{free}}(\mathbf{h}k)| / \sum_{(\mathbf{h}k)} \varepsilon \tau |F_{\text{obs}}(\mathbf{h}k)|$  where the  $\tau$  set of reflections is omitted from the refinement process. 10% of the data were included in the  $\tau$  set for calculation of  $R_{\text{free}}$  and not included in refinement. Values in parenthesis are for the highest resolution shell.

**FIG.\_2C**



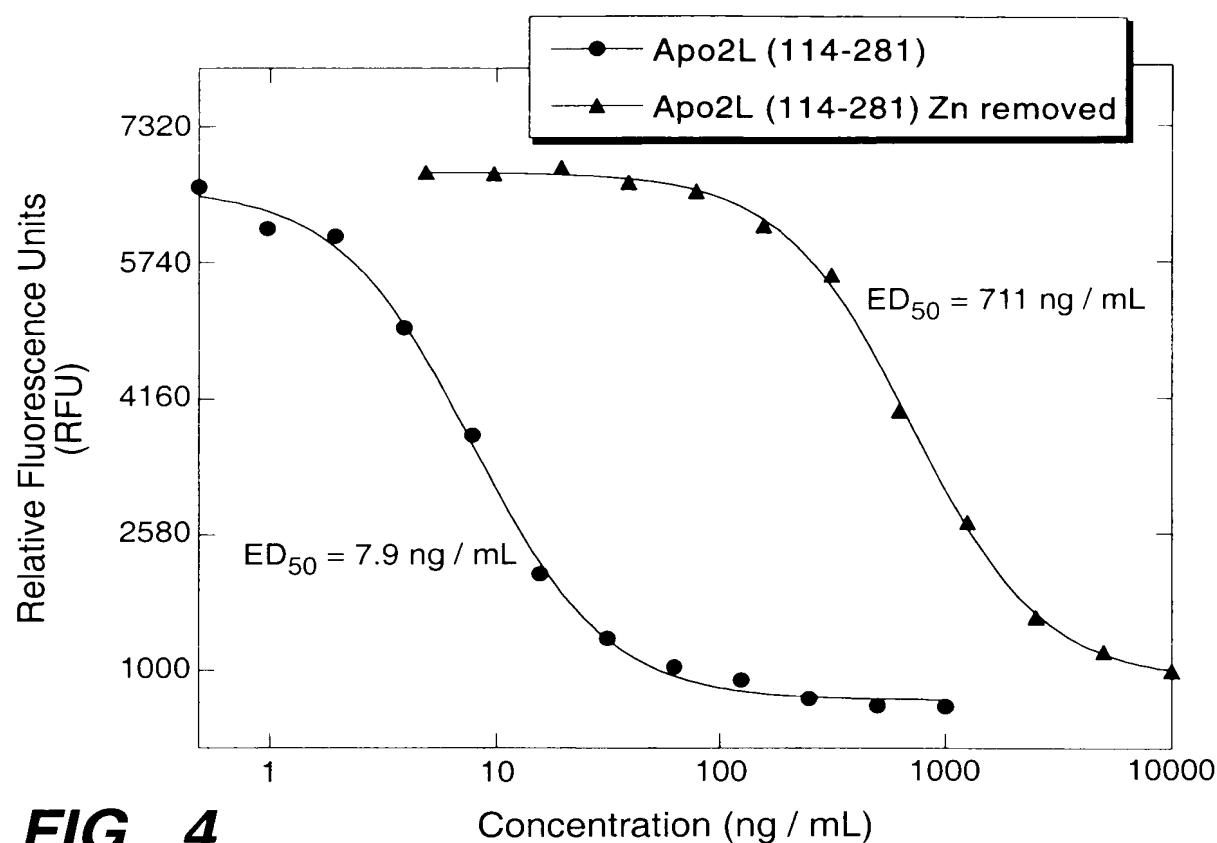
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	A		A'		B'	
	121	130	140	150	160	170
Apo2L	RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWE	SSRSRSGHSFLSNLHLR				
TNF- $\beta$	KPAAHЛИGD		PSKQNSLLWRANTDRAFLQDGFSLS			
TNF- $\alpha$	KPVAHVAN		PQAEGQLQWLNRANALLANGVELR			
CD40L	QIAAHVISE		ASSKTTSVLQWAEKGYYTMSNNLVLE			
FasL	RKVAHLTGK		SNSRSMPLEWEDTY.GIVLLSGVKYK			
RANKL	QPFAHLTIN		ATDIPSGSHKVSLSSWYHDR.GWAKISNMTFS			
	B		C		D	
	180	190	200	210		
Apo2L	NG.ELVIHEKGYYIYSQTYFRFQEEIKENTKNDKQM	VQYIYKYTSYPD				
TNF- $\beta$	NN.SLLVPTSGIYFVYSQVVFS	KGKAYSPKATSSPLYL	AHEVQLFSSQYPF			
TNF- $\alpha$	DN.QLVVPSEGLYLIYSQVLFKGQG	CPSTHVL	LTHTISRIA	SYQT		
CD40L	NGKQLTVKRQGLYYIYAQVTFCS	NREASSQAPFIASL	CLKSPG			
FasL	KG.GLVINETGLYFVYSKVYFRGQ	SCNNLPLSHKVYMRNSKYPQ				
RANKL	NG.KLIVNQDGFYLYANI	CFRHETSGD	LATEYLQLM	VYVTKTSIKIPS		
	E		F		G	
	220	230	240	250	260	
Apo2L	PILLMKSARNSCWSKDAE	YGLYSIYQGGIFEL	KENDRIF	SVTNE		
TNF- $\beta$	HVPLLSSQKMVPGLQE	PWLHSMYHGA	AFQLTQGDQL	STHTDGI		
TNF- $\alpha$	KVNLLSAIKSPCQRETPEGAEAKP	WYEPIYLGGV	FQLEKGDR	LSAEINRP		
CD40L	RFERILLRAANTHSSAKP	CGQQSIHLGGV	FELQPGASV	FVNVTDP		
FasL	DLVMMEGKMMMSYCTTGQ	MWARSSYILGAV	FNLTSADHLY	VNVSEL		
RANKL	SHTLMKGGSTKYWSGNSE	FHFYSINVGGFFKL	RSGEEISIEVSNP			
	H					
	270	280				
Apo2L	HLIDMDHE.ASFFGAFLVG					
TNF- $\beta$	PHLVLSPS.TVFFGAFL.					
TNF- $\alpha$	DYLLFAESGQVYFGIIAL.					
CD40L	SQVSHGTG.FTSFGLLKL.					
FasL	SLVNFEES.QTFFGLYK..					
RANKL	SLLDPDQD.ATYFGAFKVR					

**FIG.\_3**

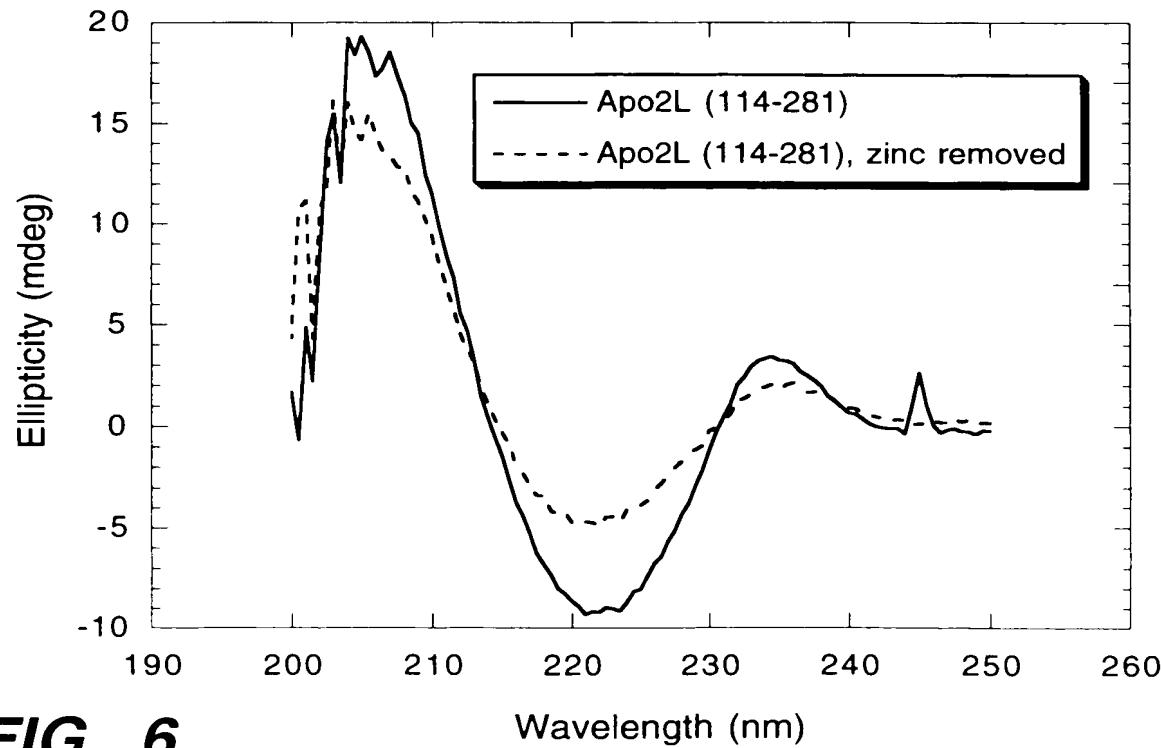
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**FIG.\_4**

### Circular Dichroic Spectra



**FIG.\_6**

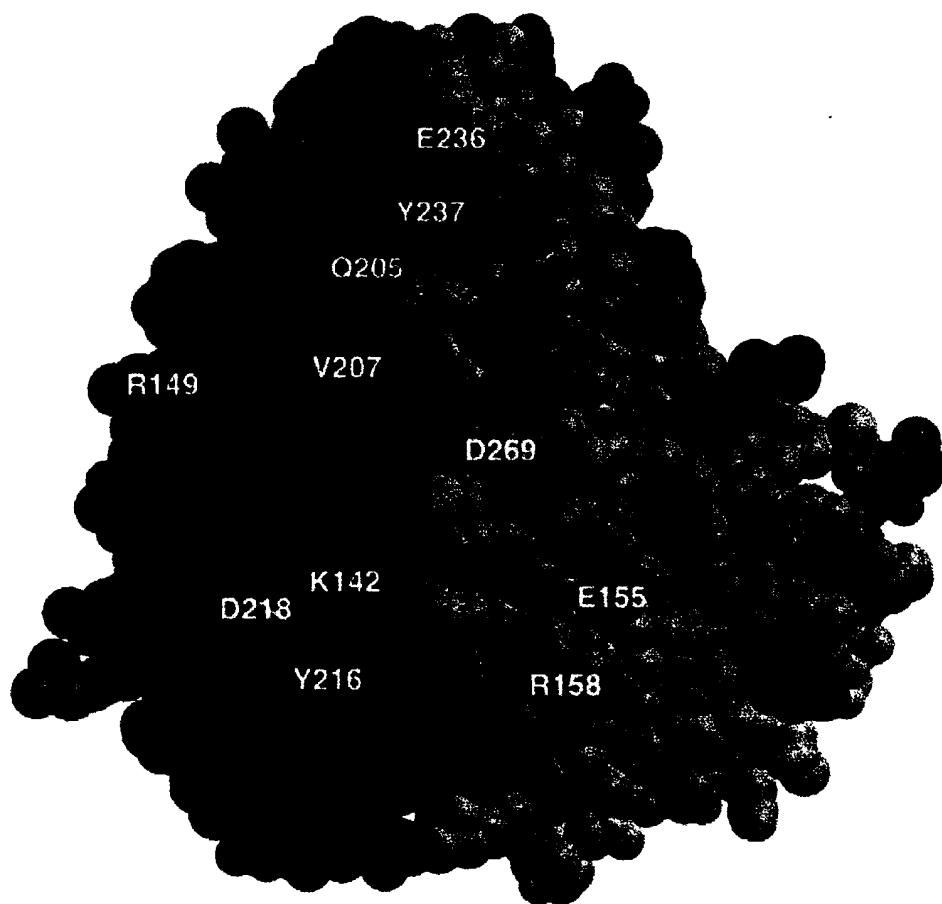
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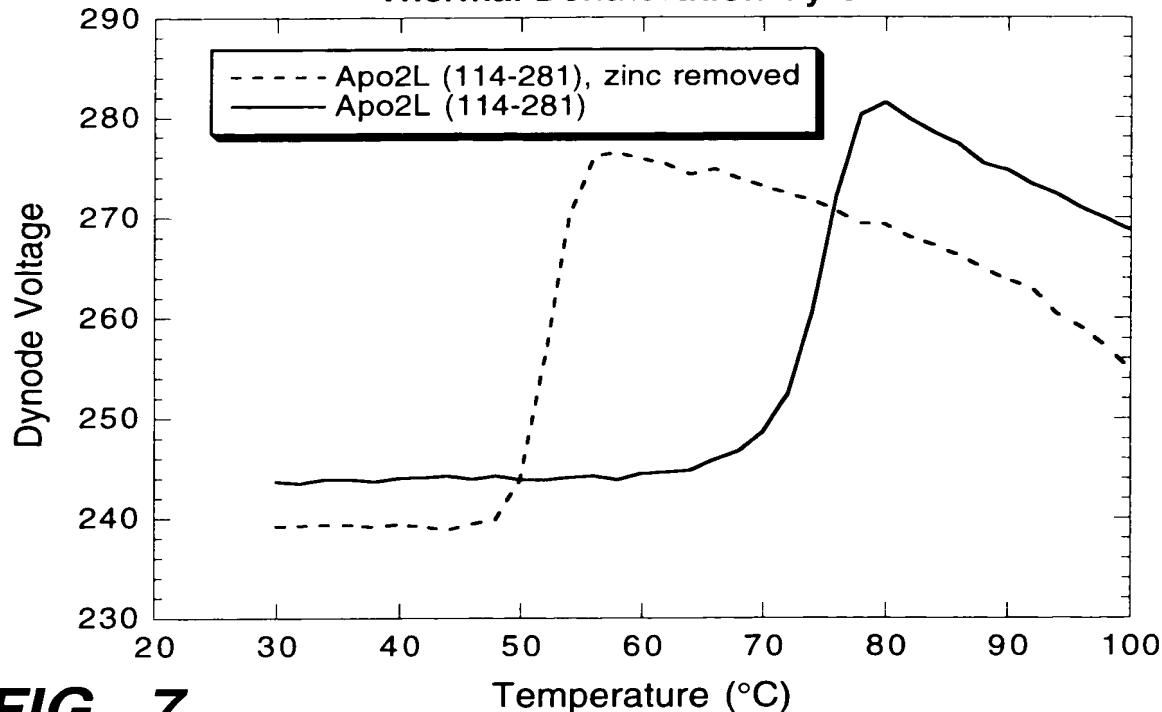
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**FIG. 5**

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### Effect of Zn Removal On Stability: Thermal Denaturation By CD

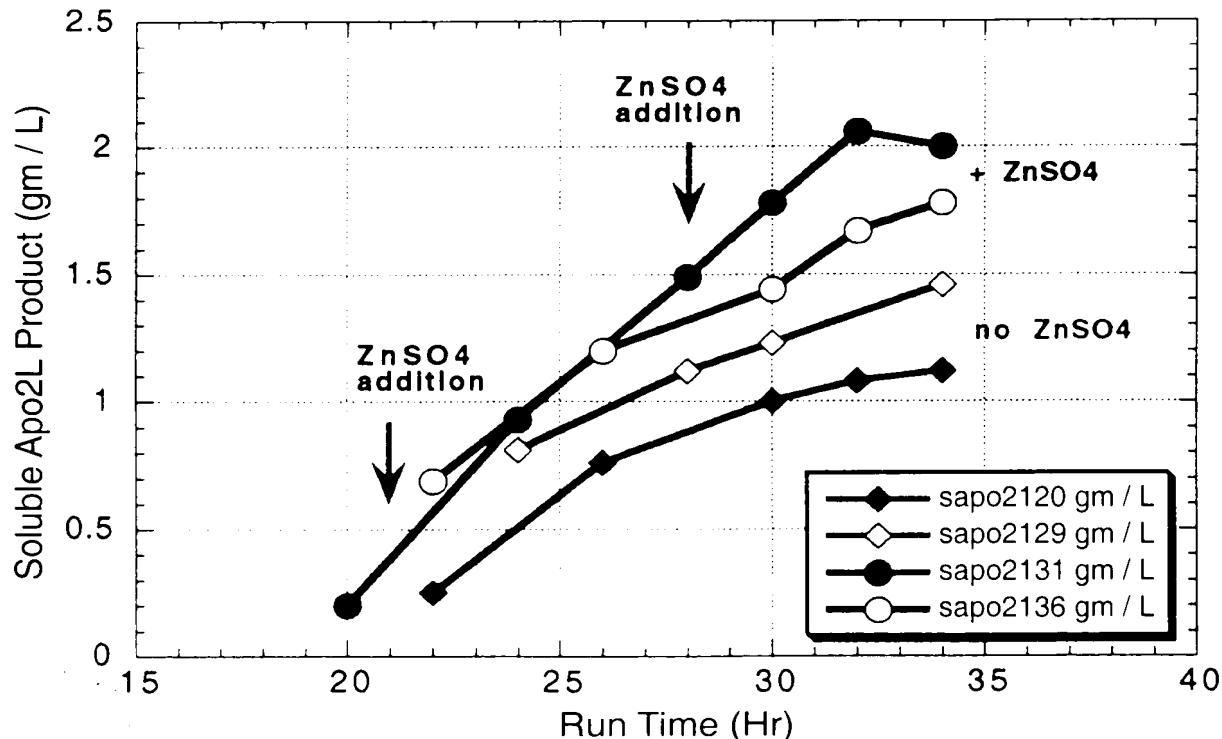


**FIG.\_7**

### Effect of ZnSO<sub>4</sub> Additions On Apo2L Product Accumulation

Production Host: 43E7

(E. coli W3110 fhuA (tonA) phoA  $\Delta$ (argF-lac) degP kanS ptr3 ompT ilvG+)



**FIG.\_8**

AP-Apo2L +/- ZnSO<sub>4</sub> gm / L

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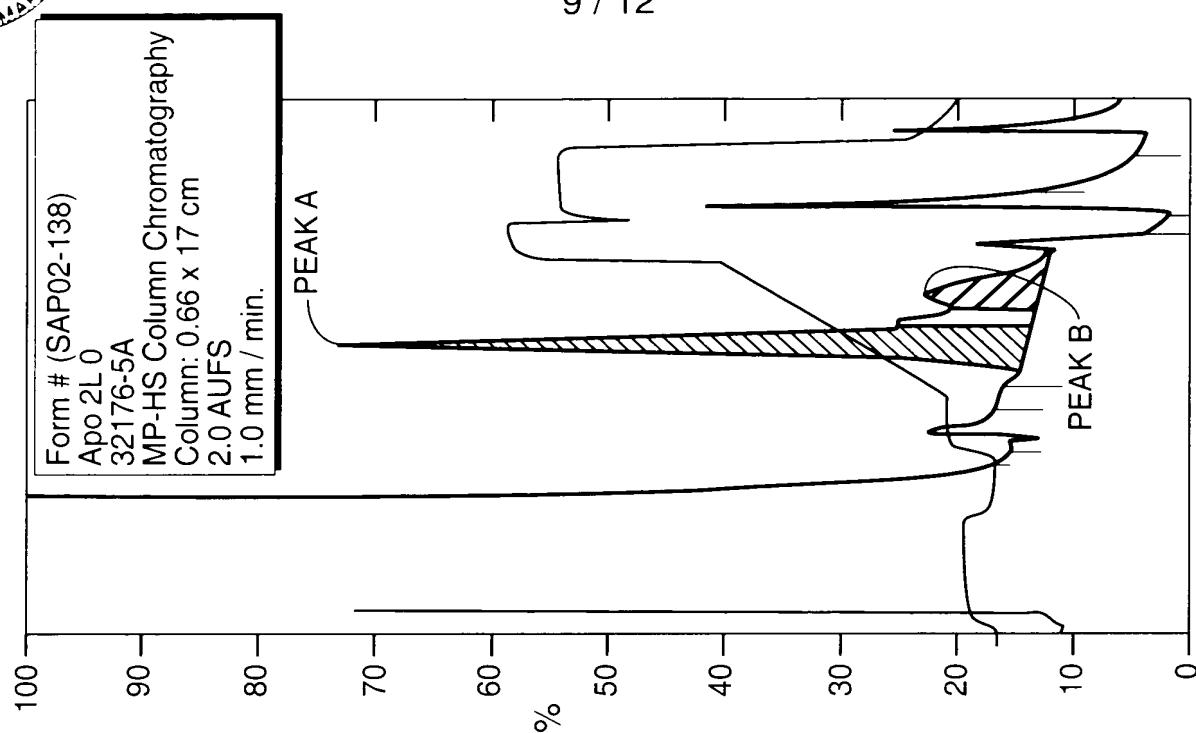


FIG.\_ 9B

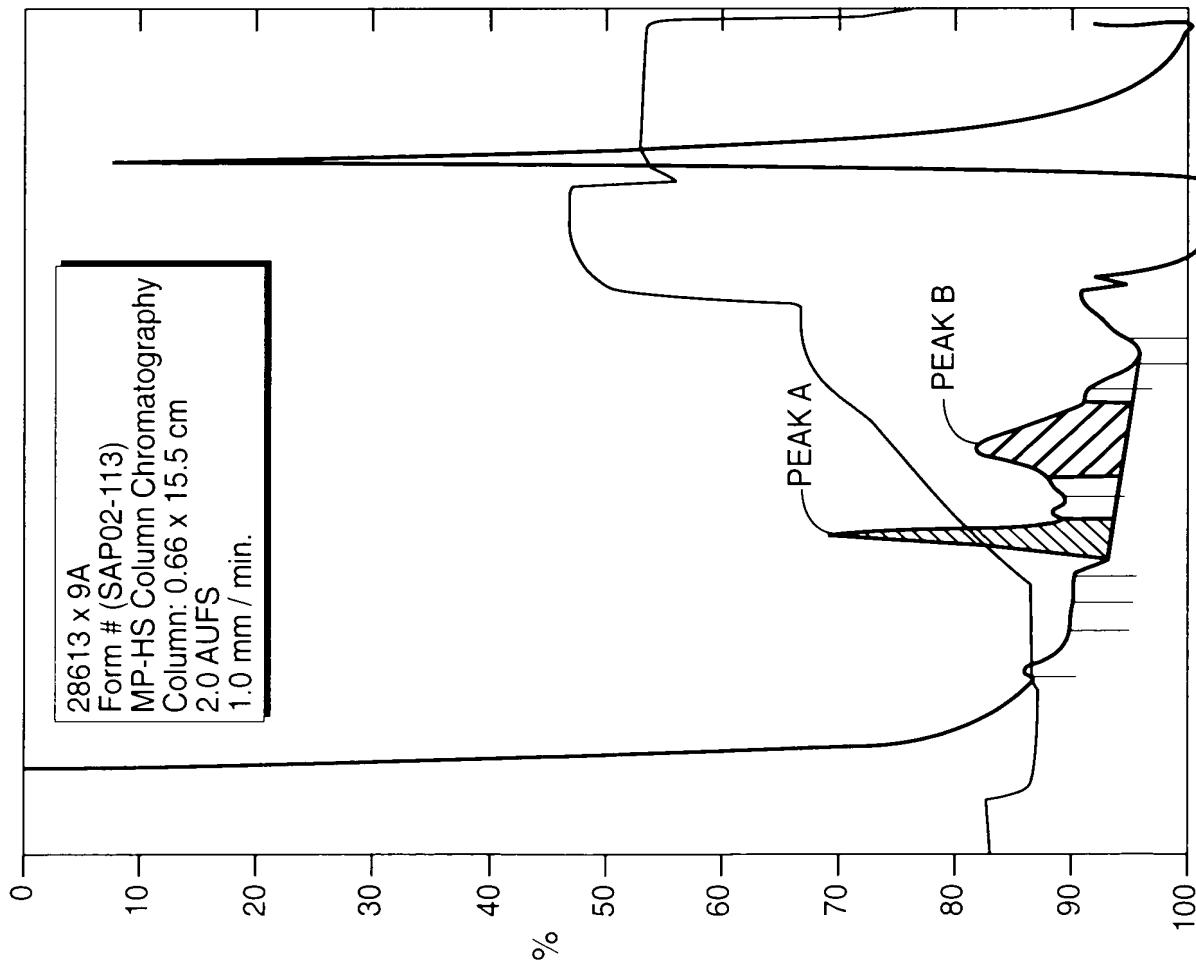
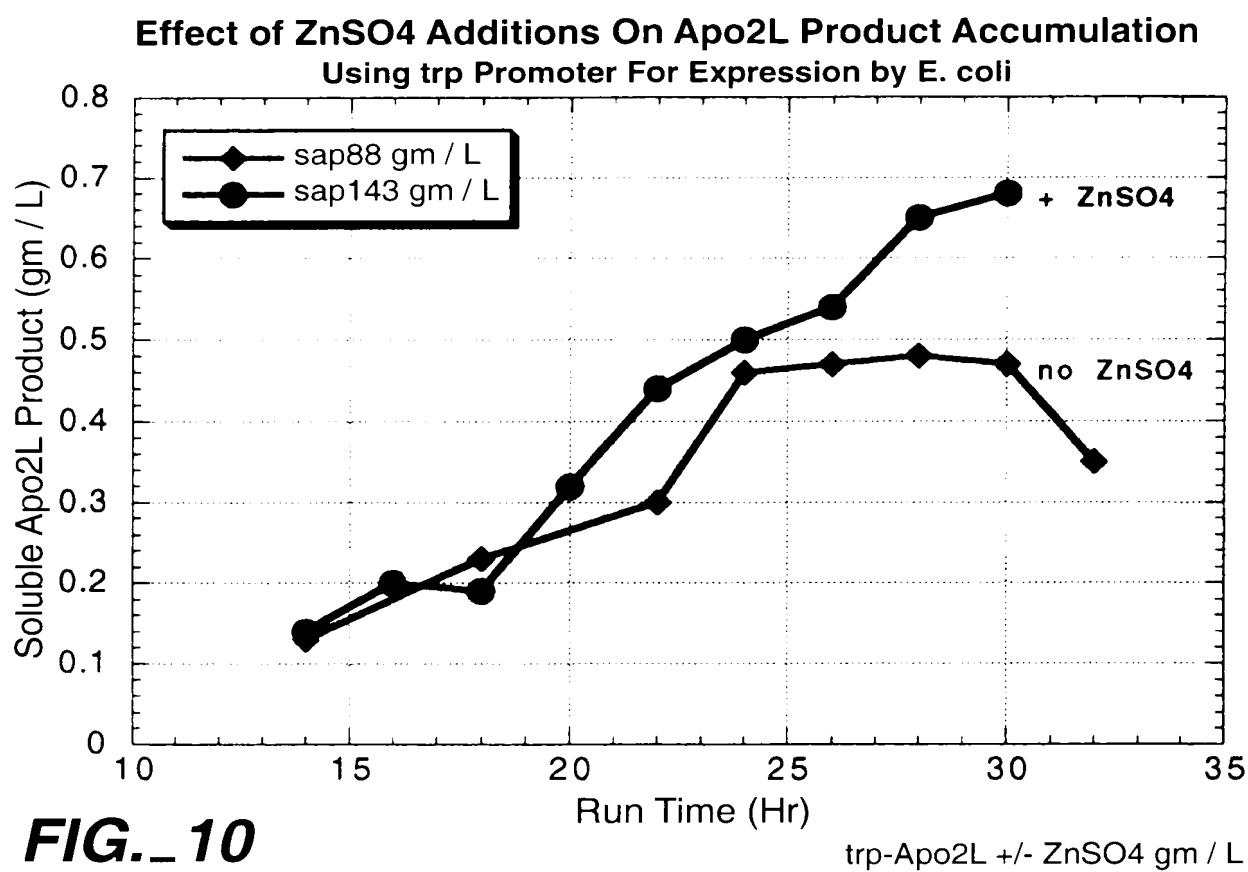


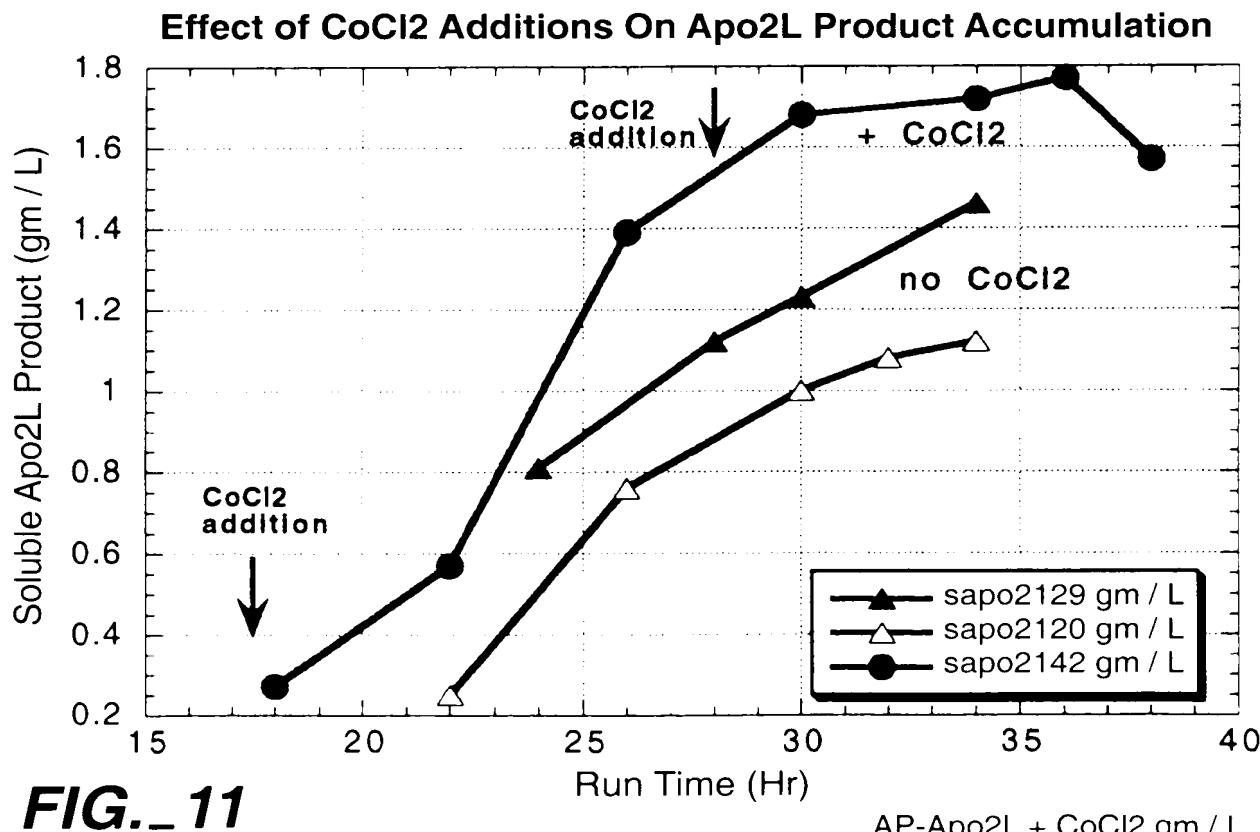
FIG.\_ 9A

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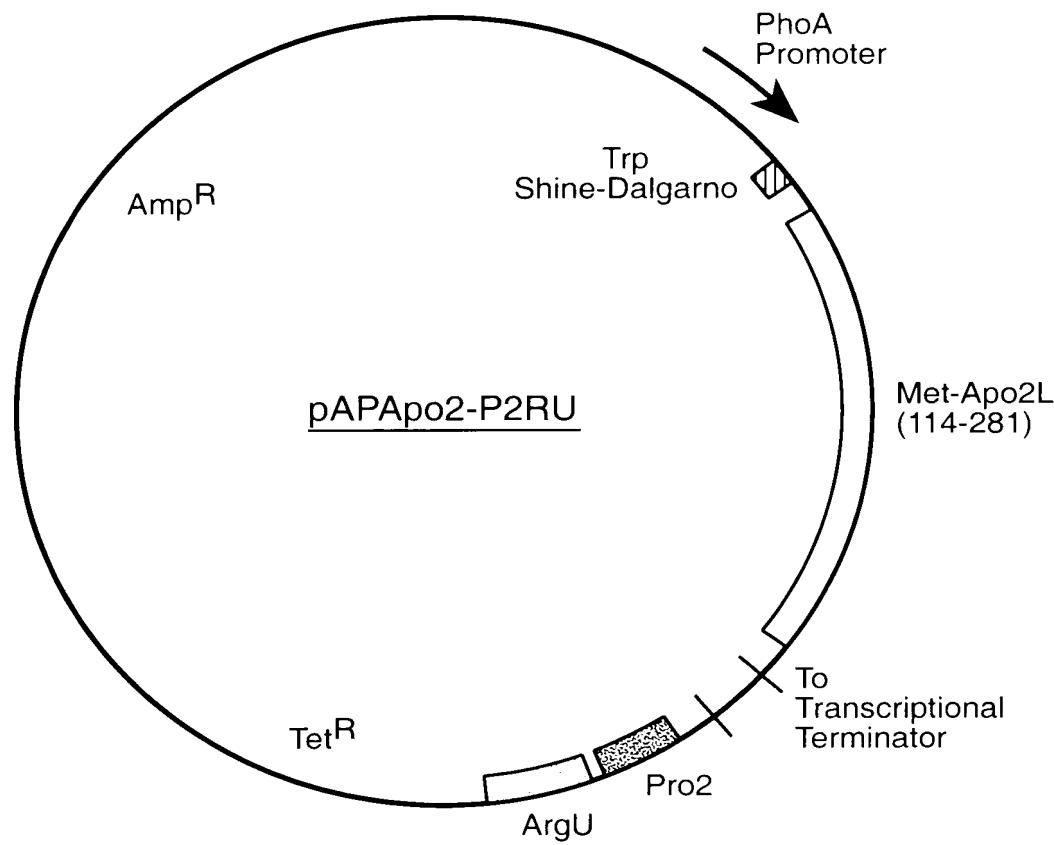
**FIG.\_ 10**



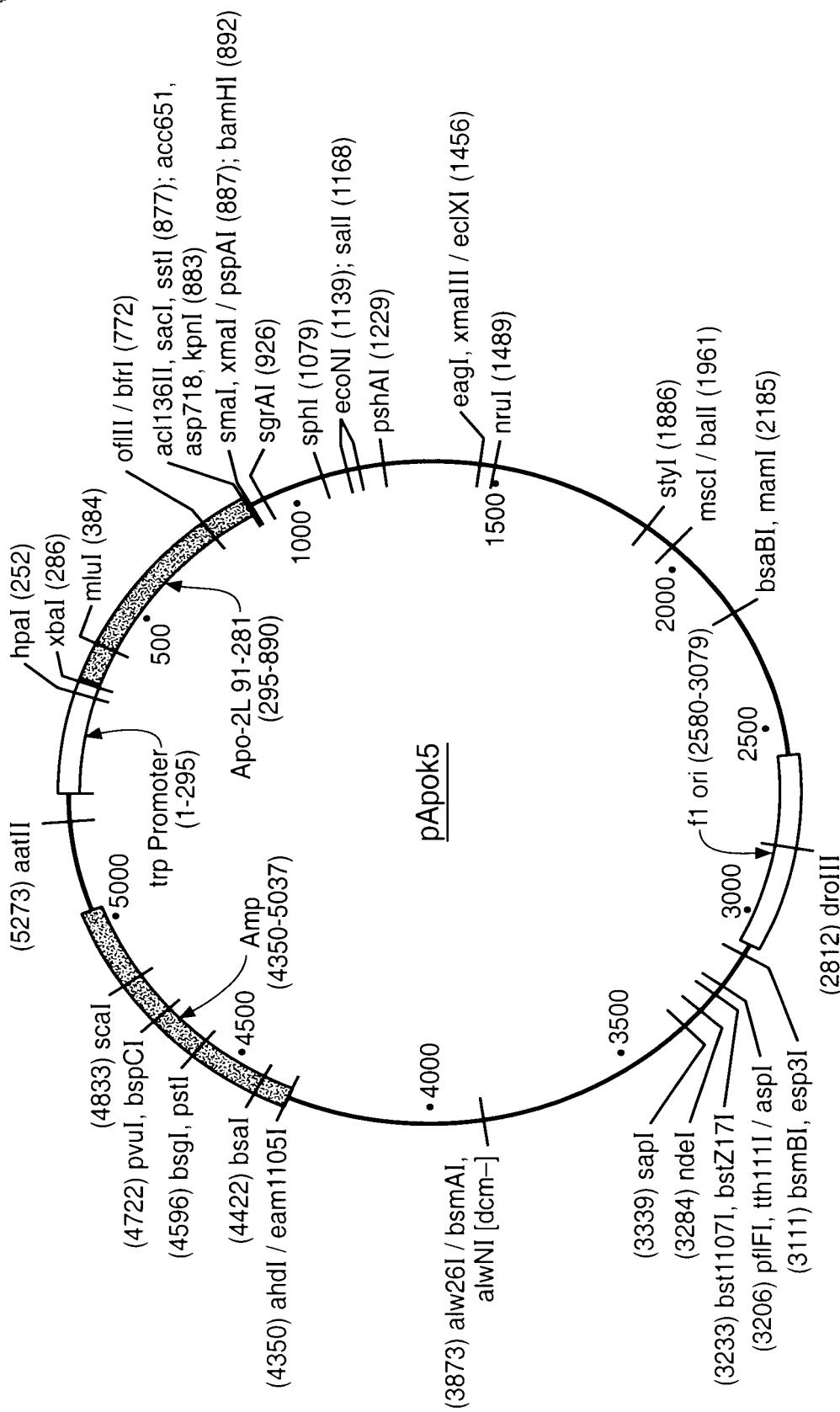
**FIG.\_ 11**



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**FIG.\_12**



**FIG. 13**